Reproductive Compatibility of Prairie and Montane Populations of *Dermacentor andersoni*

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ABSTRACT Genetic analysis of prairie and montane populations of *Dermacentor andersoni* (Stiles) originating from Alberta (AB) and British Columbia (BC), Canada, respectively, indicated limited gene flow ($N_m < 1$) and a large amount of genetic differentiation ($F_{st} = 0.49$) between the populations. The prairie population also had a greater level of genetic diversity. Mating experiments indicated that females of geographically heterogeneous crosses had similar engorgement and oviposition failure as homogenous crosses in the parental generation but that egg mass sterility was greatest for the AB♀ × BC♂ cross, intermediate for the homogenous crosses, and lowest for the BC♀ × AB♂ cross. The progeny of all crosses produced fertile eggs, and the only significant effect in the progeny generation was increased oviposition failure of the pure AB cross. Covariate analysis indicated that egg mass sterility was associated with BC males in the parental generation and that oviposition failure was associated with AB males and AB females in the progeny generation. The hazard of cumulative reproductive failure was increased with AB females in both generations, reduced for AB males in the parental generation, and increased with AB males in the progeny generation. Overall, heterogenous crosses had the greatest and least reproductive failure in the parental generation, but they were intermediate to the homogenous crosses in the progeny generation. The limited gene flow between the populations seems to have been sufficient to maintain reproductive compatibility.

KEY WORDS *Dermacentor andersoni*, population genetics, fertility, reproduction

The Rocky Mountain wood tick, *Dermacentor andersoni* (Stiles), is widely distributed in western North America (James et al. 2006). The distribution in Canada includes prairie habitat from central Saskatchewan westward to the front range of the Western Cordillera in Alberta, and montane habitat from the British Columbia border to the eastern slopes of the Coastal Mountains in British Columbia (Wilkinson 1967). *D. andersoni* from southern Alberta (prairie) and the interior of British Columbia (montane) have different sites of attachment on cattle (Wilkinson and Lawson 1965), suggesting that there might be genetic differences between tick populations in areas separated by the Rocky Mountains. The terms "prairie" and "montane" strains were applied to tick populations in these areas (Wilkinson 1967). Differences in sites of attachment among the prairie and montane ticks were further demonstrated (Wilkinson 1972), as were differences in their ability to cause paralysis (Wilkinson 1985, Lysyk and Majak 2003), and differences in body weight and developmental response to photoperiod (Pound and George 1991). Populations of *D. andersoni* also differ in susceptibility to midgut infection with *Anaplasma marginale* (Scoles et al. 2005).

D. andersoni is an important vector of several pathogens of wildlife, livestock, and humans. In Canada, the principal concern caused by this species is tick paralysis in the interior of British Columbia, resulting from feeding of montane ticks. Because ticks from prairie populations have not been shown to cause paralysis, it has been suggested that introduction of nonparalyzing forms of *D. andersoni* into British Columbia. may be a means for reducing paralysis (Wilkinson 1985). This could be accomplished by introducing prairie strains of *D. andersoni* into areas inhabited by paralyzing montane forms. The success of such a program would depend on the ability of the two populations to produce fertile offspring, without increasing disease transmission or tick populations due to hybrid vigor (Wilkinson 1985).

It is difficult to predict whether geographically isolated tick populations are reproductively isolated without conducting controlled mating studies. Populations of *Rhipicephalus sanguineus* (Latreille) from Argentina and Brazil are genetically distinct and produce sterile hybrids (Szabo et al. 2005). Mating between Australian *Boophilus microplus* (Canestrini) males and conspecific females originating from South Africa resulted in eggs with a low hatch rate, whereas the reciprocal cross resulted in eggs with a reasonably high hatch rate (Spickett and Milan 1978). However, populations of Gulf Coast tick, *Ambylomma maculatum* Koch from Kansas and Texas are reproductively

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compatible in spite of genetic differences (Ketchum et al. 2006). Populations of *Ixodes kingi* Bishopp separated by the Rocky Mountains maintain their reproductive compatibility, even though they differ in size (Gregson 1971). Wilkinson (1972) noted that progeny of a cross between *D. andersoni* males from Saskatchewan (prairie form) and females from British Columbia (montane form) produced fertile eggs, but details were sparse and no information on performance of the reciprocal cross was provided. This study was therefore conducted to quantify the degree of genetic differentiation between two isolated populations of *D. andersoni*, representing a montane population (BC) and a prairie population (AB), and to determine whether the populations are reproductively compatible.

Materials and Methods

Population genetic analysis was conducted to determine the degree of genetic differentiation between the two populations. Controlled mating studies were carried out to determine whether prairie and montane tick populations are reproductively isolated. The ticks from the two populations were crossed, and the reproductive success of the pure strain and cross-mated ticks was evaluated. Progeny of the pure strains and cross-mated ticks were reared in brother-sister matings to determine fertility of the hybrids. Analyses were conducted to determine at what stage in the reproductive process (engorgement, oviposition, and egg hatch) failures occurred and whether reproductive failures could be associated with geographic origin of the males and females.

Source of Ticks. Ticks were collected by flagging at the end of April during 2002 and 2003. AB ticks were collected from a coulee near Chin Lakes Reservoir (49° 36′ N, 112° 11′ W) and represented a prairie population. BC ticks were collected south of Kamloops in the vicinity of Walker Lake (50° 33′ N, 120° 15′ W) and were representative of a montane population. The two sites are located ≈640 km apart and are separated by mountain ranges as well as distance. Ticks that were to be used for population genetic analysis were preserved in 70% ethanol immediately after collection until genomic DNA preparations were made. Ticks used to determine reproductive compatibility were held at 10°C and 95% RH until mating experiments were conducted.

Population Genetic Analysis. Analysis of tick DNA was conducted to determine the genetic similarity between the two populations of ticks. Tick genomic DNA was prepared as described previously (Scoles 2004). Alcohol-preserved ticks were air-dried, placed individually in plastic microcentrifuge tubes, and frozen in liquid nitrogen. Then, they were ground to a fine powder by using disposable plastic pestles. Lysis solution was added and the DNA was extracted using the QIAGEN DNeasy tissue kit (QIAGEN, Valencia, CA) following the manufacturer's protocol.

An ≈1,600-base fragment of the mitochondrial 16s-12s was polymerase chain reaction (PCR) amplified using published universal tick primers 16s+1, 5′-CCG GTC TGA ACT CAG ATC AAG T-3′ and 12s-1, 5′-AAA CTA GGA TTA GAT ACC C-3′ (Norris et al. 1996). The fragment was TA cloned into pCR4-TOPO vector and transformed into TOP10 competent cells by using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Three clones were picked for each tick and grown overnight in 96-well deep-well plate with 1 ml of 2× LB media/well. Plasmid DNA was purified using Millipore 96-well plasmid miniprep kit (Millipore Corporation, Billerica, MA); DNA from each well was resuspended in 60 μl of double-distilled H_2O. The presence and size of the insert for each clone was confirmed by restriction digesting 2 μl of plasmid DNA with EcoRI and running the digestion on a 2% agarose gel. Sequencing was preformed with the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), and the products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Each clone was sequenced with three reads, one read from either end by using the T3 and T7 priming sites on the cloning vector and one read to span the gap between the end sequences by using a sequencing primer of our own design ≈390 bases downstream of the 16s+1 primer, 5′-TTA AAA TCT TAT TAC AAT ACC TTT G-3′.

Sequences were assembled using the SeqMan module of the Lasergene sequence analysis package (DNAStar, Madison, WI). The sequence for each individual tick was a consensus of at least two of the clones; additional clones were sequenced from each tick when there was ambiguity. Consensus sequences for each tick were exported from SeqMan and aligned with the MegAlign module (DNAStar). For population genetic analysis the alignment exported from MegAlign in Nexus format was imported into the DnaSP analysis package (Rozas et al. 2003). Statistical analysis of population genetic data follows the methods of Hudson and coworkers as implemented in DnaSP (Hudson 2000; Hudson et al. 1992a).

Reproductive Compatibility. *Parental Generation.* Four parental crosses were made each year using the ticks collected in the spring of that year. The homogeneous crosses were AB ♀ × AB ♂ (designated ABAB) and BC ♀ × BC ♂ (designated BCBC) and the heterogeneous crosses were AB ♀ × BC ♂ (designated ABBC) and BC ♀ × AB ♂ (designated BCAB). Each year, 30 field-collected male and female ticks for each cross were placed in cages on animals and fed until engorged females spontaneously detached. Adult ticks collected in 2002 were fed on a naïve Holstein steer during mid-August 2002. Adult ticks collected in 2003 were fed on New Zealand White rabbits during early August 2003. All animals were cared for in accordance with Canadian Council of Animal Care Guidelines by using methods approved by the Lethbridge Research Centre Animal Care Committee. Engorged females were weighed, placed in individual vials, and held at 25°C and 95% RH for oviposition.

Progeny Generation. A portion of the eggs laid by each female were collected and reared to adult on New Zealand White rabbits by using previously de-

scribed methods (Lysyk and Majak 2003). Inbred matings were made with 30 male and 30 female progeny of each cross that were placed in cages fastened to New Zealand White rabbits. Engorged females were collected and weighed.

Data Collection and Analysis. Females that engorged and spontaneously detached were handled in the same fashion for both the parental and progeny generations. Engorged females were weighed, placed in individual vials, and held at 25°C and 95% RH for oviposition. Eggs were collected three times per week, weighed, and the total weight of eggs laid per female calculated. A sample of ≈100 eggs from each female were placed in small petri dish, held at 25°C and 95% RH, and the percentage hatch was determined. Egg masses were retained and also examined to determine whether hatch occurred.

Data were collected on qualitative variables associated with failure of independent components of the reproductive process for each female placed on a host; these variables included engorgement, oviposition, and hatch. Engorgement failure was calculated for each female placed on a host, whereas oviposition failure and egg mass sterility, respectively, were calculated only for females that had successfully engorged or oviposited previously. Qualitative variables were assigned a value 1 if failure occurred or 0 if not. The quantitative variables were female engorgement weight, egg mass weight, conversion efficiency index, percentage hatch, and the milligrams of hatched eggs per female. The conversion efficiency index (CEI) was the percentage of engorged weight converted to egg and was calculated for each female as 100 × (egg mass weight)/(engorged weight) (Drummond and Whetstone 1970). The milligrams of hatched eggs per female was calculated as (mg eggs) × proportion hatched.

Qualitative variables from the parental and progeny generations were analyzed separately using Fisher exact test (Cytel Inc. 2007) to determine whether there was significant variation among crosses. Data from both years were pooled to increase the power of the tests. Analysis of variance (ANOVA) was used to determine whether each quantitative variable varied significantly among crosses. Years were treated as replicates. Means were separated using Fisher least significant difference (LSD). A second analysis was conducted to quantify the effects of the covariates: generation (G), female source (F), and male source (M) on the qualitative and quantitative variables. The generation covariate was assigned the value G = 0 for the parental generation and G = 1 for the progeny generation. The female source and male source covariates were assigned values = 1 if from Alberta and 0 if from British Columbia. Stepwise logistic regression was used to identify the effect of each covariate and two-way interaction on the qualitative variables (Proc Logistic, SAS Institute 2003). Stepwise linear regression was used to identify the same for the quantitative variables (Proc Reg, SAS Institute 2003). Yearly variation was considered random.

A final analysis was conducted to examine the effects of the covariates over the cumulative reproductive process because the logistic and stepwise regressions examined effects on individual components of the reproductive process. Engorgement, oviposition, and egg hatch were designated times 1, 2, and 3. The number of failures occurring at each time was analyzed using proportional hazards regression (Proc Phreg, SAS Institute 2003). Cox's procedure was used because it has the fewest assumptions concerning the hazard function. The model specifies the influence of covariates in terms of their effect on a hazard ratio.

Table 1. Summary statistics[a] for population genetic analysis

Pop	#	S	h	H_d	K	P_i
AB	32	26	12	0.87500	7.60887	0.00523
BC	33	18	12	0.82765*	2.58712*	0.00176*
Overall	65	35	24	0.92644	7.52019	0.00516

* Significant difference between populations, permutation test ($P > 0.0001$).

[a] #, number of sequences; S, number of segregating sites; h, number of haplotypes; H_d, haplotype diversity; K, average number of nucleotide differences; P_i, nucleotide diversity.

Results

Population Genetic Analysis. An ≈1,627 base 16s-12s mitochondrial DNA PCR product was cloned, sequenced, and analyzed from 32 AB and 33 BC ticks (GenBank accession nos. EU711283–EU711347). Sites with alignment gaps were excluded from the analysis because although some of these gaps may represent true polymorphisms, most of them occur in poly-A or poly-T regions and in many cases may represent sequencing artifacts. The total number of sites examined was 1,456. Thirty-five of these sites were polymorphic, representing 37 base changes (two sites had three variants). Of these sites, 18 were polymorphic in the AB population but monomorphic in the BC; 10 sites were polymorphic in the BC population but monomorphic in the AB population; and nine sites were polymorphic in both tick populations. Only 25 of these polymorphic sites were parsimony informative (i.e., occurred in two or more ticks). There were 24 unique haplotypes, and none of these haplotypes were shared between the two populations (i.e., there were 12 haplotypes from each population).

Summary statistics for the two populations (Table 1) indicate significant differences between populations in both nucleotide and haplotype diversity, as well as in the average number of nucleotide differences. These data also demonstrate that the AB population has a significantly higher level of genetic diversity than the BC population ($P > 0.0001$).

In addition to these summary statistics, the DnaSP program calculated a variety of population genetic statistics that are intended to provide insight into the genetic distance and gene flow between populations. For these data, these statistics included $F_{st} = 0.48738$ and $N_m = 0.53$ (Wright 1978, Hudson et al. 1992b); $N_{st} = 0.48726$ and $N_m = 0.53$ (Lynch and Crease 1990); $\delta_{st} = 0.00172$, $\gamma_{st} = 0.33790$, and $N_m = 0.98$ (Nei 1982).

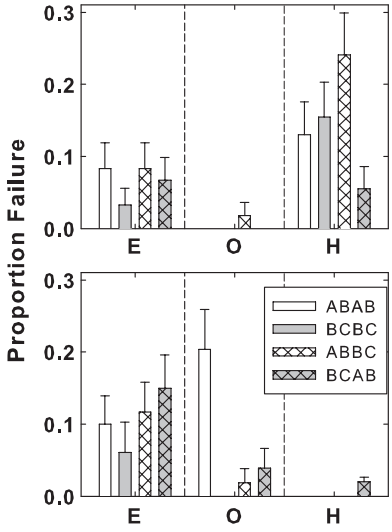

Fig. 1. Failure of tick crosses to engorge (E), oviposit (O), or have egg masses that hatched (H) in the parental (top) and progeny (bottom) generations. The first two letters of each cross designate source of females and the last two letters designate the source of males.

Reproductive Compatibility. *Parental Generation.* The percentage of females that failed to engorge ranged from 3.3 to 8.3% and did not vary significantly among crosses (Fisher statistic = 1.73, exact $P = 0.67$) (Fig. 1). Two of the engorged females were inadvertently destroyed, and only a single female of the remainder failed to oviposit. Clearly, there were no significant differences in oviposition failure among the crosses (Fisher statistic = 2.9, $P = 0.49$) in the parental generation. Egg mass sterility ranged from 5.5% to 24.1% and varied significantly among the crosses (Fisher statistic = 7.8, $P < 0.05$). Sterility was greatest for the ABBC cross, intermediate for the ABAB and BCBC crosses, and least for the BCAB cross (Fig. 1). Engorged weight varied among the crosses (Table 2) and was greatest for the BCBC cross, intermediate for the two hybrid crosses, and least for the ABAB cross. Differences in engorgement weight did not translate to significant differences in egg mass weight, CEI, percentage hatch, or milligrams of hatched eggs per female.

Progeny Generation. Percentage engorgement failure ranged from 6.1% to 15.0% (Fig. 1) but did not vary significantly among crosses (Fisher statistic = 1.7, $P = 0.64$). Oviposition failure varied among the crosses (Fisher Statistic = 15.0; $P < 0.0006$) and occurred primarily in the ABAB cross. Oviposition failure was 20% in this cross compared with 0–3.9% in the other crosses (Fig. 1). In spite of the oviposition failure, most of the resulting egg masses were fertile and egg mass sterility did not vary among crosses (Fisher statistic = 2.7, $P = 0.70$). Only one egg mass from the BCAB cross showed no evidence of hatch. As seen with the parental generation, engorgement weight was the only quantitative variable that showed significant variation among crosses. Engorgement weight was greatest for the ABAB cross, followed by the BCBC cross, with the hybrid crosses having the least engorgement weight. This also did not translate into significant differences among crosses in egg mass weight, CEI, percentage hatch or mg hatched eggs per female.

Covariate Effects. Logistic regression indicated that engorgement failure increased slightly in the progeny generation with no significant effects of male or female source, or any two-way interaction (Table 3). Oviposition failure had significant generation × male and generation × female interactions (Table 3), indicating that the effect of parental sex on oviposition failure was not consistent among generations and occurred primarily in the progeny generation. The generation × male and generation × female effects acted jointly to cause the greater degree of oviposition failure in the progeny of the ABAB cross compared with the progeny of the BCBC and heterogeneous crosses (Fig. 1). The odds of oviposition failure in the progeny generation was $(13)/(92) = 0.1413$ for crosses involving AB males compared with $1/83 = 0.0120$ for crosses involving BC males and was $12/107 = 0.1121$ for crosses involving AB females compared with $2/80 = 0.0250$ for crosses with BC females. The proportion of sterile egg masses was influenced by generation and male source (Table 3). The odds of egg masses being

Table 2. Quantitative reproductive variables for crosses of prairie (AB) and montane (BC) *D. andersoni*

	AB ♀ × AB ♂		BC ♀ × BC ♂		AB ♀ × BC ♂		BC ♀ × AB ♂		*F*	df	*P*
	n	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE			
Parental generation											
Engorged wt (mg per ♀)	55	618 ± 18a	58	670 ± 16b	55	650 ± 16ab	56	664 ± 12b	3.0	3, 216	0.03
mg eggs per ♀	54	411 ± 13	58	445 ± 11	54	436 ± 14	56	438 ± 9	1.3	3, 214	0.27
CEI (%)	54	67 ± 1	58	67 ± 1	54	68 ± 2	55	66 ± 1	0.2	3, 213	0.91
% hatch	47	89 ± 3	49	89 ± 3	41	87 ± 4	52	92 ± 3	1.2	3, 181	0.31
mg hatched eggs per ♀	47	356 ± 16	49	403 ± 17	41	395 ± 23	52	406 ± 15	1.8	3, 181	0.16
Progeny generation											
Engorged wt (mg per ♀)	54	688 ± 19a	31	652 ± 26ab	53	630 ± 20b	51	635 ± 21ab	2.7	3, 181	0.05
mg eggs per ♀	43	453 ± 25	31	452 ± 20	52	422 ± 15	49	419 ± 20	0.8	3, 167	0.49
CEI (%)	43	63 ± 3	31	69 ± 1	52	67 ± 1	49	66 ± 2	0.2	3, 167	0.16
% hatch	43	92 ± 2	31	93 ± 2	52	94 ± 2	48	93 ± 2	0.2	3, 166	0.91
mg hatched eggs per ♀	43	417 ± 25	31	423 ± 20	52	402 ± 18	48	402 ± 20	0.6	3, 166	0.63

CEI, conversion efficiency index = 100 × (mg eggs)/female wt. Thirty males and females per cross and generation were fed on hosts in each of two replicate years.

Table 3. Final parameter estimates for stepwise logistic,[a] linear,[b] and proportional hazards regressions[c]

	Engorgement failure[a]	Oviposition failure[a]	Egg mass sterility[a]	Reproductive failure[c]	CEI[b]	% egg hatch[b]
X_i						
Intercept	−2.64 ± 0.26	−5.64 ± 0.80	−1.45 ± 0.24	−1.93 ± 0.37	67.1 ± 0.6	89.3 ± 1.4
Female (F)				0.55 ± 0.22		
Male (M)			−0.78 ± 0.40	−0.47 ± 0.29		
Generation	0.58 ± 0.34		−3.38 ± 1.02	1.43 ± 0.47		3.9 ± 2.0
G × F		1.78 ± 0.69				
G × M		2.49 ± 0.80		1.63 ± 0.52	−2.3 ± 1.2	
Test statistic	$\chi^2 = 34.4$	$\chi^2 = 34.4$	$\chi^2 = 36.1$	$\chi^2 = 16.5$	$F = 3.5$	$F = 4.0$
df	1	2	2	4	1, 394	1, 361
P	0.09	<0.0001	<0.0001	<0.003	=0.06	<0.05

All nonintercept parameters significant at $P < 0.10$.

sterile was 32/189 = 0.1693 in the parental generation compared with 1/174 = 0.0057 in the progeny generation. The significant male effect on egg mass sterility indicated that sterility was reduced for crosses involving AB males compared with BC males. The odds of sterility were 11/190 = 0.0579 for crosses including AB males and 22/173 = 0.1271 for crosses including BC males.

Stepwise regression indicated no significant effects of female or male source, generation, or any two-way interaction on engorged weight ($F = 1.3$; df = 6, 406; $P = 0.24$) or egg mass weight. Mean CEI ranged from 63% to 69% (Table 1). Stepwise regression identified a significant male × generation interaction on CEI (Table 3) that indicated 2.3 ± (1.2) % lower CEI for crosses involving AB males compared with BC males in the progeny generation. Percentage of egg hatch in fertile egg masses was high, ranging from 87% to 94%, and varied among generations only (Table 3), increasing 4% in the progeny generation compared with the parental (Table 2).

Cumulative Effects. The hazard of reproductive failure over the entire reproductive process was influenced by generation, female source, male source, with a significant generation × male interaction (Fig. 2; Table 3). The effect of female source was independent of generation and indicated greater hazard when AB females were in the cross compared with BC females. The odds of reproductive failure for crosses involving AB females was 56/183 = 0.306, whereas that for crosses involving BC females was 32/180 = 0.178.

The effect of male source on the hazard of reproductive failure was dependent on generation as indicated by the significant generation × male interaction. During the parental generation, the hazard for AB males was lower than for BC males with the odds of reproductive failure equal to 19/99 = 0.19 and 30/90 = 0.33, respectively. This was reversed in the progeny generation as the hazard for AB males was greater than for BC males and the odds of reproductive failure equalled 29/91 = 0.32 for crosses with AB males compared with 10/93 = 0.12 for crosses involving BC males. The hazard of reproductive failure was greater in the progeny generation compared with the parental for crosses involving AB males, whereas the hazards of reproductive failures were lower in the progeny generation than in the parental generation for crosses involving BC males.

Discussion

Population genetic analysis suggests that there is a very high degree of genetic differentiation between these two tick populations. The F_{st} value (0.48738) is well above the level (0.25) that Wright considered to represent "very great" genetic differentiation (Wright 1978) and the estimate for N_m (the number of migrants per generation) between the two populations is less than one ($N_m = 0.53$), suggesting that there are very low levels of gene flow (Hudson et al. 1992b). Both the N_{st} statistic of Lynch and Crease (1990) and the δ_{st} and γ_{st} statistics of Nei (1982) also suggest similarly high levels of differentiation and low levels of gene flow between these two populations. These re-

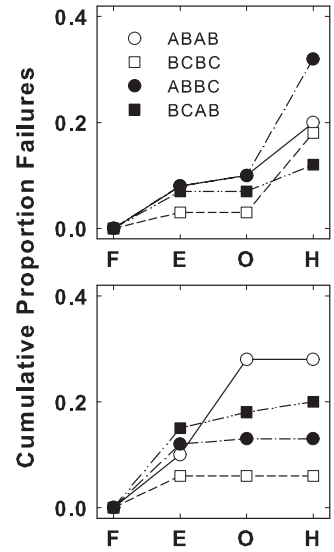

Fig. 2. Cumulative reproductive failure of tick crosses during the reproductive process (F, flat ticks; E, engorgement; O, oviposition; and H, hatch) in the parental (top) and progeny (bottom) generations. The first two letters of each cross designate source of females and the last two letters designate the source of males.

sults confirm the assertions of Wilkinson (1967) that montane and prairie populations are largely isolated genetically.

The level of nucleotide diversity of the AB ticks is significantly higher than the nucleotide diversity of the BC ticks. This suggests that the prairie population may be ancestral to the montane population; however, analysis of a much larger data set that includes a larger number of ticks collected from a wider diversity of sites in each habitat type will be needed to test this hypothesis. A larger population genetic study of this species will also need to include nuclear markers in addition to the mitochondrial sequence data analyzed here.

The quantitative variables showed few differences among the crosses. Only engorgement weight varied among the crosses within each generation, but the variation could not be associated with male or female source. The CEI was depressed for crosses involving AB males in the progeny generation, but the effect was slight. This was similar to the observation that CEI was slightly reduced in one heterogeneous cross of geographically separated populations of Gulf Coast tick, *Ambylomma maculatum* Koch (Ketchum et al. 2006). Percentage of hatch did not vary among crosses, but it increased slightly in the progeny generation.

Reproductive incompatibility as measured by the qualitative variables was incomplete because fertile progeny were produced by a large proportion of females in each heterogeneous cross. We did not observe complete reproductive failure of heterogeneous crosses as has been noted with other tick species (Szabo et al. 2005). Egg hatch failure, or egg sterility, occurred primarily in the parental generation. The appearance of egg sterility effect was asymmetric in the heterogeneous crosses and depended on the source of males. Crosses that included AB males had reduced egg mass sterility compared with those that included BC males, and the homogenous crosses were intermediate. Asymmetry in the male effect on heterogeneous crosses has been noted in *Boophilus* ticks (Spickett and Malan 1978). Egg sterility did not occur to any appreciable extent in the progeny generation, indicating that effects were not carried over to the next generation. Oviposition failure occurred mainly in the progeny generation, and it was confined to the inbred ABAB cross. This is consistent with earlier results indicating oviposition failure was 0.18–0.24 greater for field-collected AB ticks than BC ticks (Lysyk and Majak 2003). The significant male and female effects in the progeny generation suggest there may be some inherent or latent incompatibility factor associated with AB ticks that is expressed when inbred matings occur. This was clearly not expressed in the progeny generations of the hybrid crosses or BC pure cross.

Overall, reproductive failure was associated with a female effect that was consistent among the generations, and a male effect that was not. The female effect indicated that reproductive failure was increased when crosses included AB females. The male effect varied among generations. As indicated earlier, BC males were associated with increased egg mass sterility in the parental generation, and AB males were associated with increased oviposition failure in the progeny generation. The net result was that the homogenous crosses had reproductive success intermediate to the heterogenous crosses in the parental generation resulting in the asymmetry noted earlier. In the progeny generation, the homogenous BC cross had the lowest reproductive failure, the homogenous AB cross had the greatest reproductive failure, and the heterogeneous crosses were intermediate. These results suggest that it may be possible to use AB ticks in a release program to genetically reduce the incidence of paralyzing ticks in BC because hybrids were fertile and the progeny did not have greater vigor compared with the BCBC cross.

This study indicates that *D. andersoni* from two geographically isolated populations have a limited amount of reproductive incompatibility in spite of the apparently very large amount of genetic differentiation between the populations. Differentiation between the populations is likely the result of genetic drift; these two populations were separated by the Rocky Mountains, which limits gene flow between them. Drift, coupled with the selection pressures imposed by the need to adapt to environmental conditions that differ between the regions may have resulted in local adaptation. Because *D. andersoni* does not parasitize birds, the limited gene flow is most likely the result of infrequent movement of adults ticks carried across the east-west mountain passes on large animal hosts, as suggested by Wilkinson (1967). However, this limited amount of migration seems to have been sufficient to maintain reproductive compatibility in spite of significant genetic differentiation. A similar situation occurs with *Ixodes kingi* where AB specimens are larger than BC specimens, have different hosts, are separated by the Rocky Mountains, but still produce viable progeny when crossed (Gregson 1971).

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